PROTEIN PATTERN CHANGES IN THE DEVELOPING SEEDS OF BEANS (*Phaseolus vulgaris* L.)[1] /

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Resumen

La formación de proteínas de reserva durante el desarrollo de las semillas fue estudiado mediante electroforesis en geles. El contenido proteínico de las semillas en desarrollo aumenta gradualmente presentando un período rápido de depósito que comienza alrededor de 18 días después de la floración.

A los 30 días una proteína semejante a vicilina predomina y continuará así a través del período de maduración. La proteína tipo legumina parece sintetizarse en un estado posterior del desarrollo de la semilla, puesto que no fue encontrada en el día 30, pero sí en el 50, 60 y en semillas totalmente maduras.

Introduction

In legume seeds the cotyledons form the bulk of the seed and synthesize most of the protein. In the developing cotyledon there are two phases of growth, an initial one of intensive cell division followed by a longer period of growth by cell expansion. During expansion growth, 95% of the protein is synthesized (2, 14). Early investigations on the course of seed development concentrated mainly on the gross changes in nitrogen, starch-sugar, and metabolic aspects such as respiratory activity (12). The accumulation of storage protein in developing pea seeds had been followed by Danielsson (4) and the origin and transfer of nitrogen and carbon compound to developing pods and seed had been examined (16).

The storage proteins of legume seeds are generally of two types, vicilin-like and legumin-like (5, 10). Danielsson (4) found that vicilin was synthesized prior to legumin in ripening pea seeds; this pattern has been confirmed in *Vicia faba* (19). Millerd *et al.* (14) found that synthesis of storage protein began early in seed development. Hall *et al.* (8) reported that in *Phaseolus vulgaris* the synthesis of storage protein was initiated when the seed attained 10 mm length. Hill and Breidenbach (9) studing soybean storage proteins described that the 2.2S sedimenting proteins predominated at very early stages of development and decreased proportionately throughout maturation. The 7.5S and 11.8S components appeared to be synthesized later in maturity and in larger amounts than the 2.2S proteins. Electrophoretic studies revealed temporal differences in the accumulation of the three components of the 7.5S fractions. The 11.8S sedimenting fraction appeared throughout seed development as a homogeneous protein which accumulated in the seed with a time course similar to that of the total 7.5S protein fraction.

The patterns of protein accumulation in developping seeds of various legume species have received considerable attention. It is frequently difficult to integrate much of the published data, mainly because of the great variation in growing conditions. This paper describes the changes in protein pattern observed after electrophoretic separation of extracts from seeds during development.

Materials and methods

Plant material

Seeds of *Phaseolus vulgaris* L. cv. Goiano precoce were germinated as described before (11). After 7

1 Received for publication in April 18, 1985.

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days of germination, the seedlings were transfered to plastic pots containing a complete Hoagland's nutrient solution and cultured with continuous aeration. Seeds were harvested according to their physiological age (days after flowering). The seeds were freeze-dried and stored in a vaccum dessicator until used. Only uniform material was collected and no account was taken of the position of the pod on the plant. The growth chamber was controlled with a day temperature of 30°C and a night temperature of 22°C. A combination of 4 000 ft-c of fluorescent and incandescent light was provided for 16 hours each day with the remaining hours in the dark.

Protein samples

The freeze-dried samples were carefully ground in a chilled mortar with Tris-NaCl buffer [0.2 N NaCl in 0.05 M tris (hydroxymethyl aminomethane)], pH 8.0. The homogenates were centrifuged at 23 000 x **G** for 30 min at 0°C and the resulting supernatants were then dialyzed overnight against excess Tris-NaCl buffer in a cold room. Protein content was determined by Lowry's method (13) and the volume of each sample necessary to give approximately 300 μg of protein for electrophoresis was calculated.

Disc electrophoresis

Polyacrylamide gel collums (7%) were prepared as described by Davis (6). Electrophoresis using about 0.3 mg protein sample was usually carried out for 50 min in a tris –glycine buffer with a current of 5 mA per gel column. Detection of the separated protein components was achieved by staining the gels for 2 hours with 0.5% aniline blue black in 7% acetic acid and destained by diffusion with several changes of 7% acetic acid.

Sample length and weight

Sample length and fresh weight were taken immediately after the samples were collected. Dry weight was measured after the sample was dried in a 70°C oven for 3 days. Five pods were collected each time for the length and weight determination.

Results

Weight and lenght changes of developing pods and seeds

The changes in weight and length of developing seeds are shown in Figure 1-A. During the first 2 weeks, the seed growns very slowly except in length, which starts to increase constantly after flowering.

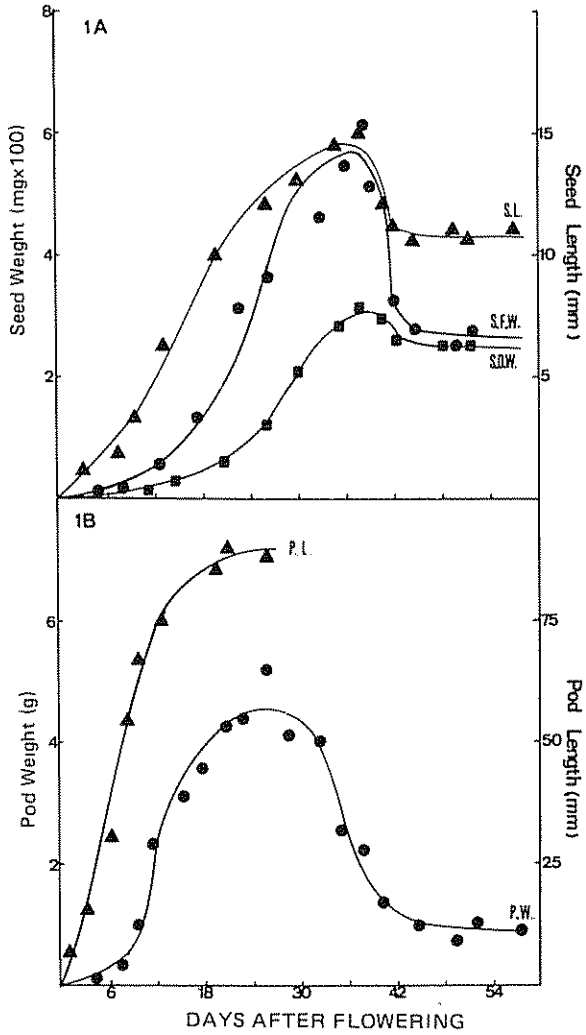

Fig. 1. A: Weight and length changes during seed development of *Phaseolus vulgaris* L. cv. Goiano precoce. (S.L.: Seed length; S.F.W.: Seed fresh weight; S.D.W.: Seed dry weight).

B: Weight and length changes of pod during *Phaseolus vulgaris* L. cv. Goiano precoce seed maturation. (P.L.: Pod length; P.W.: Pod weight).

The seed length reaches a maximum at about 35 days and then declines until day 42. The seed fresh weight starts to increase greatly 2 weeks after flowering. Thirty-seven days after flowering they achieved their maximum value. The fresh weight decreased sharply until day 45 and was there after constant.

The dry weight of the seed increased in a similar way as the fresh weight. It also reached its maximum value at about day 37. While the rate of fresh weight declined rapidly, the dry weight of the seed decreased only slightly and reached a constant value. The seeds were fully mature by day 60 when many of them

were loose in the pod and the remaining showed some degree of abscission.

The changes in weight and length of developing pod are shown in Figure 1-B. The pods attained their maximum weight of pod was obtained about 24 days after flowering. Marked shrinkage of the pod tissues became apparent between day 30 and 35. By day 45, the pods were completely dried and a constant pod weight was obtained since then.

The percentage of dry matter and water content are shown in Figure 2-A. While the percentage of dry matter was initially low it increased with increasing fresh weigth. The most rapid dry matter percentage increase seems to occur between day 35 and day 40. After this period, dry matter was constant at about 90% of the total fresh weight, until full maturation.

Protein content changes

The increase in the Tris-NaCl extractable proteins of the developing seed was determined by biochemical analysis (Figure 2B). The changes closely parallel that in dry weight, except that protein synthesis is maintained until the seed is completely mature. Initially, the protein content of seeds increased gradually, with a rapid period of deposition starting at about 18 days after flowering until near day 37. From this day, on, until full maturation, the protein content still increased but at a relatively slow rate. Throughout development, protein forms an increasing part of the total dry weight of the cotyledon.

Protein pattern changes

The proteins of flower, pod and developing seed were each separated by disc gel electrophoresis. The results are shown in Figure 3. Four diffused and fast moving bands were found in the extract of flowers which contain very small pods (gel 1). The 3-day-old and 5-day-old pods (with very small seed inside) also contained rapidly migrating bands but several intermedial banks were also present (gels 2,3). The 9-day-old seeds contained mainly high to medium mobility bands but a relatively low mobility band (Rm = 0.28) was also present (gel 4). This band predominated over all other bands during the seed development from day 13 to day 23 (gels 5, 6, 7). At this stage several other low mobility bands and 3 medium mobility bands were also observed. At day 30, the concentration of Rm 0.28 band sharply decreased, and one of the medium mobility protein (Rm = 0.52), the vicilin-like protein (7), became dominant (gel 8). This protein predominated thereafter throughout maturation. At day 50 other low mobility proteins (e.g. legumin-like protein, Rm = 0.10) were observed and at day 60, all the protein bands detected in the dry mature seed were observed (gels 9, 10).

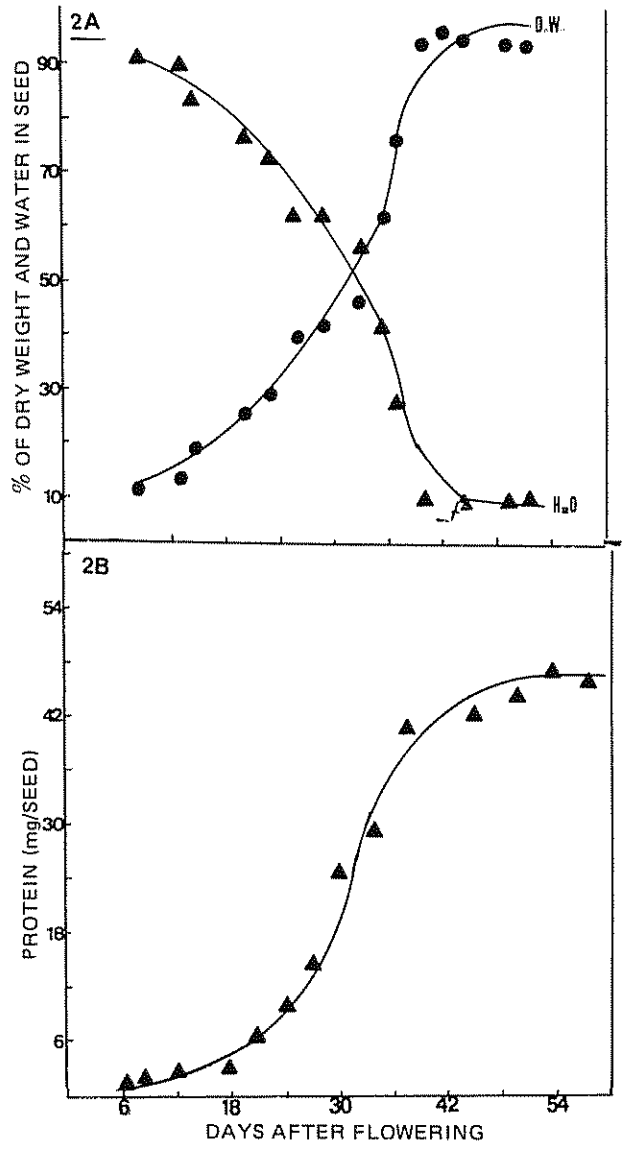

Fig. 2. A: Percentage of dry weight (D.W.) and water in developing seed of *Phaseolus vulgaris* L. cv. Goiano precoce.

B: Changes of Tris-NaCl extractable protein during seed development of *Phaseolus vulgaris* L. cv. Goiano precoce.

Discussion

The developing seed of legumes has been generally recognized as a important tool for studies on the quantitative and qualitative regulation of protein synthesis (1, 14). However, before this possibility can be fully utilize the temporal pattern of protein accumulation during seed development should be established. The general pattern of fresh weight, dry weight and protein accumulation changes of the

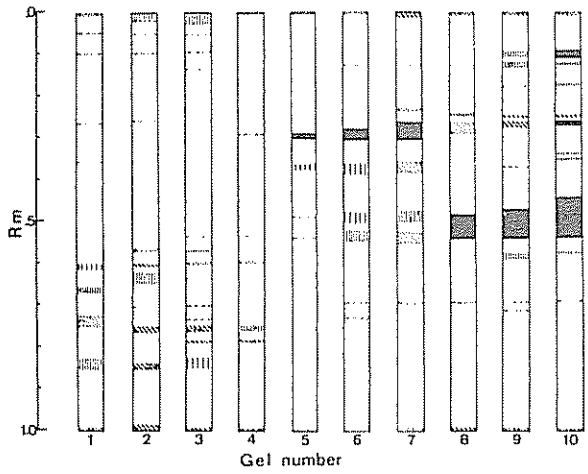

Fig. 3 Disc gel electrophoretic patterns of Tris-NaCl extractable proteins in developing seed of *Phaseolus vulgaris* L. cv. Goiano precoce. Gel 1: Flower extract; Gel 2: 3-day-old pod; Gel 3: 5-day-old pod; Gel 4: 9-day-old developing seed; Gel 5: 13-day-old developing seed; Gel 6: 18-day-old developing seed; Gel 7: 23-day-old developing seed; Gel 8: 30-day-old developing seed; Gel 9: 50-day-old developing seed; Gel 10: 60-day-old mature seed

maturing seeds were studied in order to correlate them with the electrophoretic patterns of the proteins of the developing seeds. The weight increase of the cotyledons follows the usual sigmoid curve, with the rapid phase beginning approximately just after the liquid endosperm is exhausted, and decreasing when cell expansion is completed. Pod weight increased rapidly between about 7 and 18 days after flowering suggesting that much of the substrate available for fruit formation is conducted into the pod.

The pattern of accumulation of protein was similar to those found by other investigators (9, 7, 18). The amount of protein present increased rapidly during the phase of rapid increase in dry weight. The composition of the storage fraction of legumes changes during the course of accumulation of protein during seed development and these changes suggest that the rate of synthesis of individual proteins differ and may even vary during ripening. Several investigators have employed electrophoresis of total seed protein or globulin to follow the changes which occur in individual proteins (8, 9). The results presented demonstrate that changes do occur and they give some indication of the electrophoretic components involved. However, different globulins may have similar electrophoretic mobilities and the possible presence of both monomeric and dimeric forms of a protein (3, 17) prevents further interpretation of the data. Also, the high concentration of vicilin-like protein (10), which accumulates during the last stage, rapidly masks other proteins, readily detectable after electrophoresis on acrylamide gels. Results obtained by the use of the ultracentrifuge alone have similar interpretation problems (9). In order to follow the changing protein pattern during seed development, proteins must be monitored by one of their unique characteristics, for example, immunological determinants, as reported by Millerd and Spencer (15).

Summary

The formation of storage protein during seed development was followed by gel electrophoresis. The protein content of developing seed increased gradually with a rapid period of deposition starting at about 18 days after flowering. At day 30, the vicilin-like protein became predominant and continued so thereafter throughout maturation. Legumin-like protein appeared to be synthesized at a later stage of seed development since it was not observed in the day 30 seed but was revealed at day 50, day 60 and full-maturation seeds.

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